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METABOLIC FATE OF THE SHORT-ACTING PERIPHERAL NEUROMUSCULAR BLOCKING AGENT STERCURONIUM IN THE RAT, AS RELATED TO ITS ACTION

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Abstract—Among a number of synthetic derivatives of conessine, a steroid of natural origin, the mono-quaternary compound conessine ethoiodide (stercuronium) was outstanding as a very short-acting neuromuscular blocking agent of the non-depolarizing type. After intravenous administration of [^{14}C]labelled stercuronium the short duration of action was found to be directly related to the rapid decline of the blood level below a critical concentration. During the neuromuscular blockade, excretion of radioactivity in urine and bile, although substantial, was still too small to explain the rapid decline of the blood level. Whole-body autoradiography showed rapid uptake of stercuronium radioactivity in liver and kidneys to be the main factor determining the short action. Both excretion and distribution studies show prolonged retention of part of the administered radioactivity dose in the organism. Excretion data obtained for some rats reveal different half-lives for the urinary and biliary excretion of radioactivity, which suggests the existence of at least two separate storage pools. Judging from the long-term distribution studies, these pools are probably located in the liver and kidneys. Analytical studies gave no evidence for biotransformation of stercuronium in the rat.

DURING the past few years our chemists synthesized a large number of derivatives of connessine, a compound extracted from the bark of *Holarrhena antidysenterica L.*, a plant growing in India and Central Africa.

The synthetic derivatives were investigated for various pharmacological activities, including e.g. neuromuscular blockade. Both mono- and bisquaternary conessine derivatives were examined in a search for a short-acting muscle relaxant without the disadvantages of either the depolarizing drugs such as succinylcholine or the non-depolarizing drugs like *d*-tubocurarine. Stercuronium, a very short-acting neuromuscular blocking agent of the non-depolarizing type appears to meet these requirements.

The present article deals with aspects of the metabolic fate of stercuronium in the rat, as encountered in studies undertaken to obtain more insight into the factors determining the short duration of action.

MATERIALS AND METHODS

Labelled compound

The experiments were performed with stercuronium iodide, labelled with ^{14}C as shown in Fig. 1.

The synthesis was based on coupling of 3β-dimethylamino-cona-3,5-dienine with

ethyl iodide-^{14}C. The specific radioactivity of the compound obtained was 3·2 mc/m-mole. Chemical and radiochemical purity were confirmed by thin-layer chromatography.

Animal experiments

In all experiments the radioactive drug was injected as a solution in physiological saline into the jugular vein.

During the neuromuscular blockade artificial respiration was maintained via a tracheal cannula.

To obtain information on the relationship between blood level and action, male rats (TNO-CPW/Wu, spf), 250–300 g, were anesthetized with phenobarbital sodium (150 mg/kg, i.p.) and pretreated with heparin (500 I.U.). Via a cannula, shunted in the carotid artery the circulating blood was passed through a special counting device for the continuous measurement of radioactivity. In the same experiment the curare-like

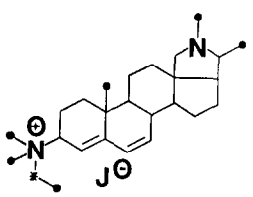

FIG. 1. Structure of stercuronium iodide-^{14}C.

activity was followed by recording the contractions of the gastrocnemius muscle under electric stimulation of the sciatic nerve. In some experiments moreover a cannula was inserted into the common bile duct and the elimination of radioactivity in the bile studied. The distribution of the labelled compound was studied in male rats, weighing 150–175 g, by the macroautoradiographic method described by Ullberg.[1] After freeze-drying, whole-body sections (30 μ) taken in a freezing room (−15°) with the aid of a microtome (Jung Modell K) were brought into contact with Kodak Industrex D photographic plates. The autoradiograms obtained were printed for illustration purposes, so that white corresponds to a high level of radioactivity.

The excretion pattern of the radioactivity was studied in male rats, weighing 150–180 g, which immediately after recovery from the neuromuscular blockade, were placed in metabolic cages for the separate collection of urine, feces, and respiratory carbon dioxide.

In order to understand the importance of the biliary elimination of radioactivity the excretion experiments were repeated in rats whose bile ducts had been ligated the day before. The decrease in fecal elimination was taken as a criterion of biliary elimination under normal conditions.

In the excretion experiments we observed that in the period of muscle relaxation substantial amounts of radioactivity were already excreted in small, spontaneously produced amounts of urine. The quantitative importance of this early excretion process was studied more closely in female rats of ±200 g with a cannulated urethra.

Analytical procedures

Urine and bile were analyzed for unchanged stercuronium and radioactive metabolites by extraction and thin-layer chromatography. The extraction was performed with butanol or dichloroethane at pH 12. The residue of the evaporated extracts (Rotavapor) was taken up in a small volume of methanol. The solution obtained was spotted on activated aluminium oxide thin-layer plates (Dünnschicht Fertigplatten Merck, Typ E, F 254). Chromatographic separations were carried out using the following solvent systems:

(a) methylisobutyl ketone (5)–methanol (15)–acetic acid (5), stercuronium showing an R_f value of about 0·95;
(b) benzene (65)–pyridine (20)–acetic acid (15), stercuronium showing an R_f value of about 0·50.

Stercuronium was located as a brown spot by allowing iodine vapour to act on the dried plates, followed by spraying with concentrated sulfuric acid and Dragendorff's reagent. The chromatographic behaviour of stercuronium was found to be highly influenced by coextracted impurities in the biological material. Retention at the place of spotting and tailing was observed. To facilitate interpretation non-radioactive stercuronium as well as an external and as an internal reference compound were employed (added to the methanol solution before spotting). Moreover, a procedure consisting of elution and respotting was applied.

Radioactivity measurements

Samples of urine and other fluids containing radioactivity were mixed with 10 ml of the scintillation cocktail according to Bray.[2] The radioactivity in the expired air was measured by binding the carbon dioxide into an ethanolamine-containing scintillation mixture,[3] of which 15-ml aliquots were measured. Fecal radioactivity was determined according to the method of Mahin and Lofberg,[4] samples of the material to be measured being destructed directly in counting vials, followed by addition of an appropriate scintillation cocktail.

All samples were counted in a Packard Tri Carb Liquid Scintillation Spectrometer 3375 using the method of external standardization.

The blood level was continuously monitored by passing the blood through a circuit of plastic scintillating tubing (Nuclear Enterprise NE 102A tubing), placed in a light-tight housing between two photomultipliers (Philips PW 4210) and connected to additional counting equipment, including a ratemeter-recorder combination.

Thin-layer chromatograms were scanned for radioactive spots with a Berthold Dünnschicht Scanner LB 2720.

For the partial quantification of the autoradiographic results small circular samples were taken from the dried whole-body sections and the adhering Scotch tape by means of a cork-bore and counted while suspended in the Bray scintillation mixture. This method resembles that of Wang and Jones[5] in the counting of [^{14}C]radioactivity absorbed on filter paper, who showed that the geometry of such samples relative to the photomultipliers does not significantly affect counting efficiency.

Dialysis experiments

In order to study the binding of stercuronium-^{14}C to serum constituents 1 ml of rat serum and 20 ml of a buffer solution (0·1 M citrate–phosphate, pH = 7) were

mixed in a dialysis bag (Visking 27/32). The bag was immersed in a solution of 6 μg/ml of stercuronium-^{14}C in the same buffer. After standing at 37° for 24 hr the radioactivity of the solution in both compartments was determined in disintegrations per minute and the binding percentage calculated according to the formula:

$$\% \text{ bound} = \frac{\text{dis./min/ml dialysis bag–dis./min/ml dialysis bath}}{\text{dis./min/ml dialysis bag}}.$$

RESULTS

Simultaneous determination of blood level and action

Figure 2 represents a concomitant measurement of the blood level of labelled stercuronium and the neuromuscular blocking activity after i.v. administration.

The injection was promptly followed by a rapid increase of the level in the circulating blood and by neuromuscular blockade. Once the maximum was reached the course of the blood curve was remarkable. Roughly two phases are to be distinguished, viz. a very rapid fall, followed by a more gradual decline to the baseline. Below a certain,

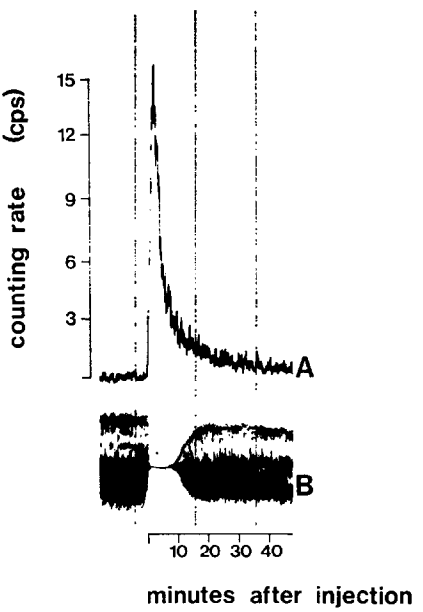

Fig. 2. Time course of the radioactivity in blood (a) and the simultaneously recorded contractions of the gastrocnemius muscle (b) in the male rat after i.v. administration of 5 mg/kg of stercuronium-^{14}C.

from animal to animal fairly reproducible, blood concentration, which was calculated at $\pm 0{\cdot}1$ μg equiv. of stercuronium per ml, the muscle contractions gradually returned. Obviously the short duration of the action of stercuronium is directly related to the rapid decline of the blood concentration below the effective value.

The results of a comparable study also concerned with excretion of the radioactivity in bile are shown in Fig. 3. The bile was collected over consecutive 5-min periods and the excretion percentages were plotted cumulatively.

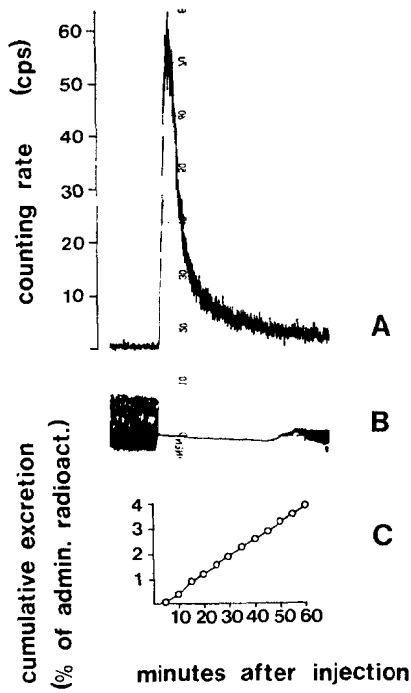

FIG. 3. Simultaneous time course of the radioactivity in blood (a), the contractions of the gastrocnemius muscle (b), and the cumulative excretion of radioactivity in bile (c) in the male rat after i.v. administration of 10 mg/kg of stercuronium-^{14}C.

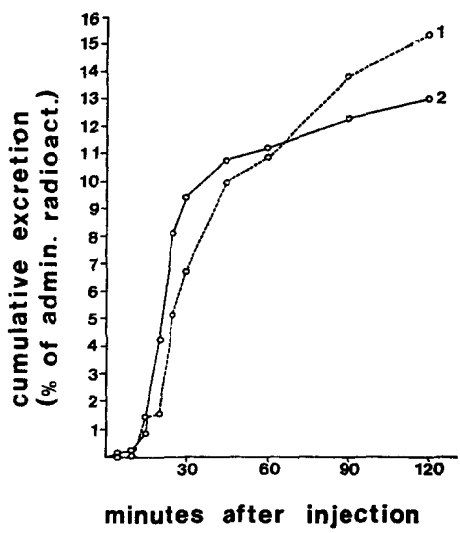

FIG. 4. The cumulative excretion of radioactivity in the cannulated urine of two female rats after i.v. administration of 10 mg/kg of stercuronium-^{14}C.

The biliary excretion of the stercuronium proved substantial, but over the period of study it was quantitatively not of such importance to account for the rapid fall of the blood level. The same conclusion is valid for the urinary excretion studied in cannulated female rats.

Figure 4 shows a substantial elimination of radioactivity in the urine over the first 30 min, about the period of neuromuscular blockade. However, even in combination with the biliary elimination this process does not provide any quantitative explanation for the initial rapid decline of the blood level. On the other hand, both cholestasis and ligation of the renal arteries led to some extension of the period of action of the drug. In blood level studies this appeared related to a retarded decline of the blood level of radioactivity below the critical level, manifesting itself especially after the initial rapid fall had taken place.

Autoradiographic distribution studies during the period of action

In order to explain the rapid decline of the blood level, we studied more closely the fate of the stercuronium radioactivity in the rat. Representative examples of the distribution of the radioactivity during the period of action are given in Figs. 5, 6 and 7: four min after injection the major portion of the radioactivity was already located in the liver and kidneys (Fig. 5), a feature even more outstanding at the subsequent intervals. The figures in Table 1 were obtained by a quantitative evaluation of the distribution as far as the concentration of radioactivity in liver and blood were concerned, using the whole-body sections as described in the experimental part.

The characteristic time course of the blood level found in the above studies is confirmed. The rapid uptake of radioactivity in the liver led to a maximum concentration at about 30 min after administration. At about the same time the first indications of radioactivity in the intestine were observable (Fig. 7), which is probably related to biliary excretion and would seem to be the major cause of the subsequent decline of the liver concentration.

TABLE 1. QUANTITATIVE EVALUATION OF THE DISTRIBUTION OF RADIOACTIVITY BY DIRECT MEASUREMENT OF TISSUE SAMPLES TAKEN FROM WHOLE-BODY SECTIONS OF RATS DOSED WITH 10 mg/kg, i.v., OF LABELLED STERCURONIUM

Time interval after administration (min)	Radioactivity of identical tissue samples (counts/min)*	
	Heart blood	Liver
2	32·4	206·8
4	15·3	270·0
8	6·2	272·4
15	7·7	346·6
30	4·5	520·0
60	4·6	400·9

* Each figure is the mean of the measurements of three samples taken out of different whole-body sections from one animal.

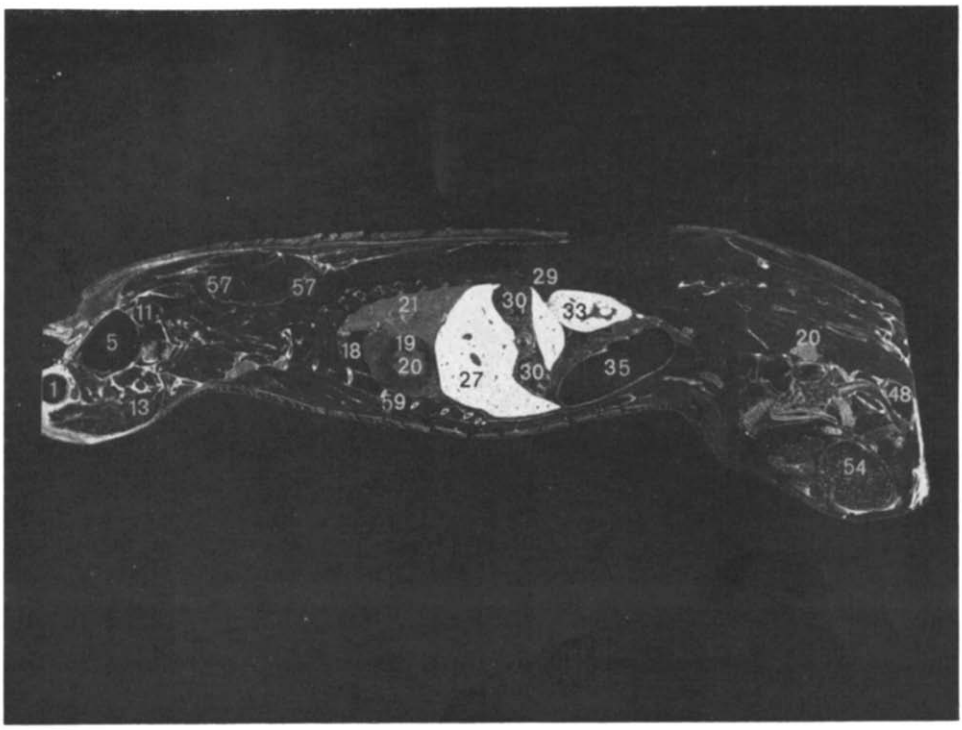

FIG. 5. Distribution of radioactivity in a rat at 4 min after i.v. administration of 10 mg/kg of stercuronium-^{14}C.

1. eye
5. cerebrum
8. choroidal plexus
10. hypophysis
11. cerebellum
13. tongue
15. salivary gland
18. thymus
19. heart
20. blood
21. lung
22. spinal marrow
23. vertebra
25. intervertebral disk
27. liver
29. spleen
30. stomach
33. kidney
34. ileum
35. cecum
36. colon
48. feces
51. skeletal muscles
54. testis
57. connective tissue
58. meninges
59. ribs
61. skin
62. epiphyseal disk

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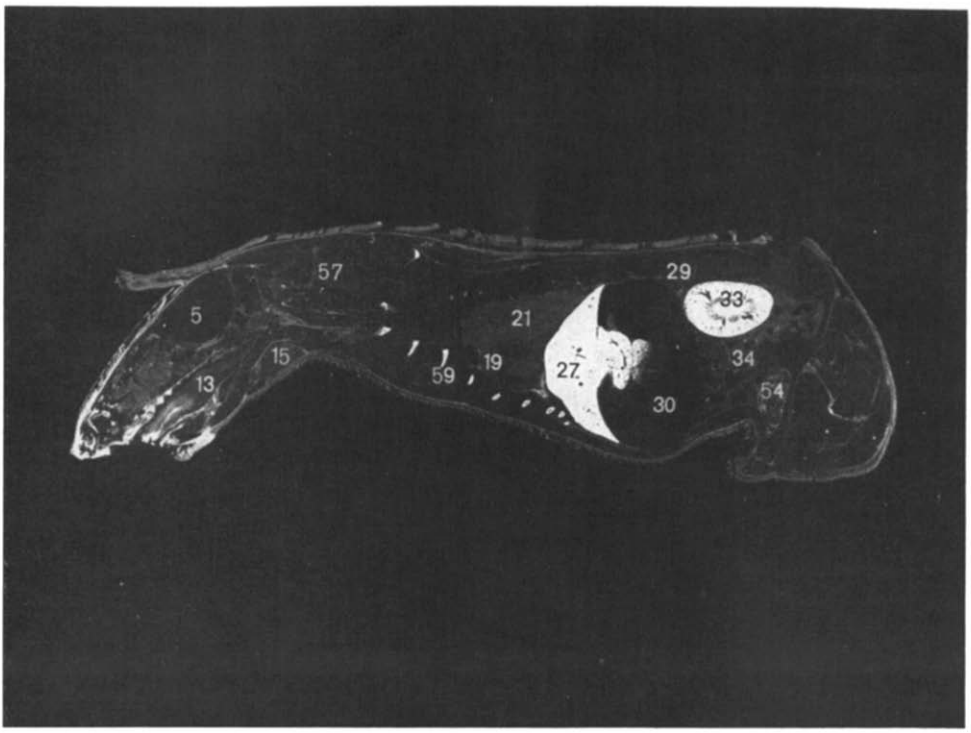

FIG. 6. Distribution of radioactivity in a rat at 8 min after i.v. administration of 10 mg/kg of stercuronium-^{14}C. (See legend to Fig. 5.)

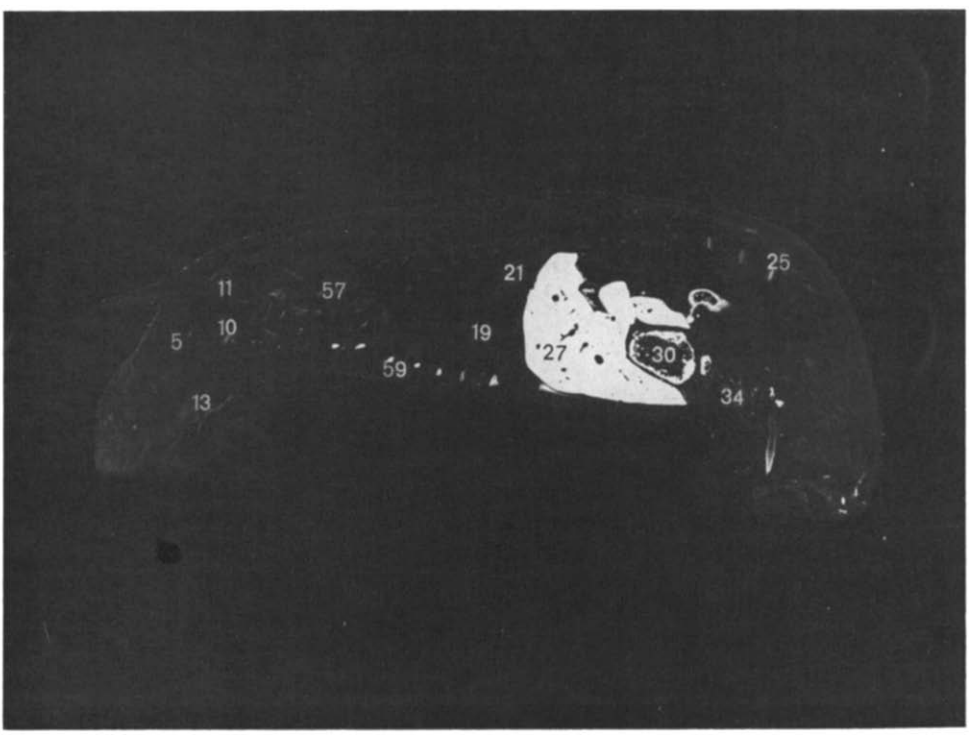

FIG. 7. Distribution of radioactivity in a rat at 60 min after i.v. administration of 10 mg/kg of stercuronium-^{14}C. (See legend to Fig. 5.)

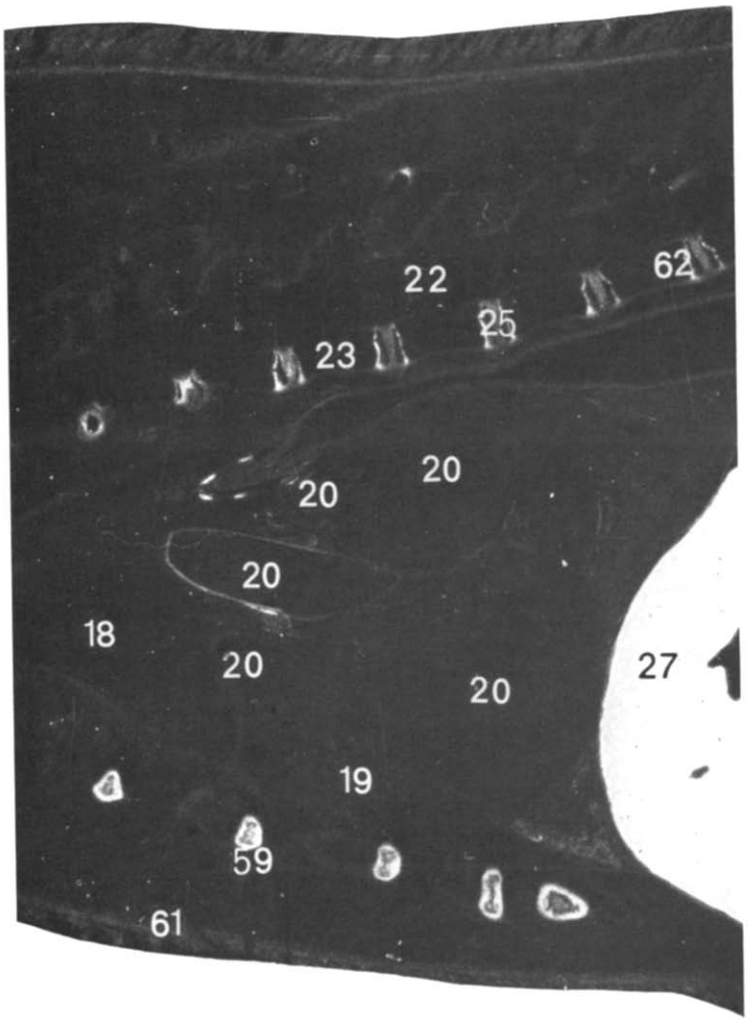

FIG. 8(a).

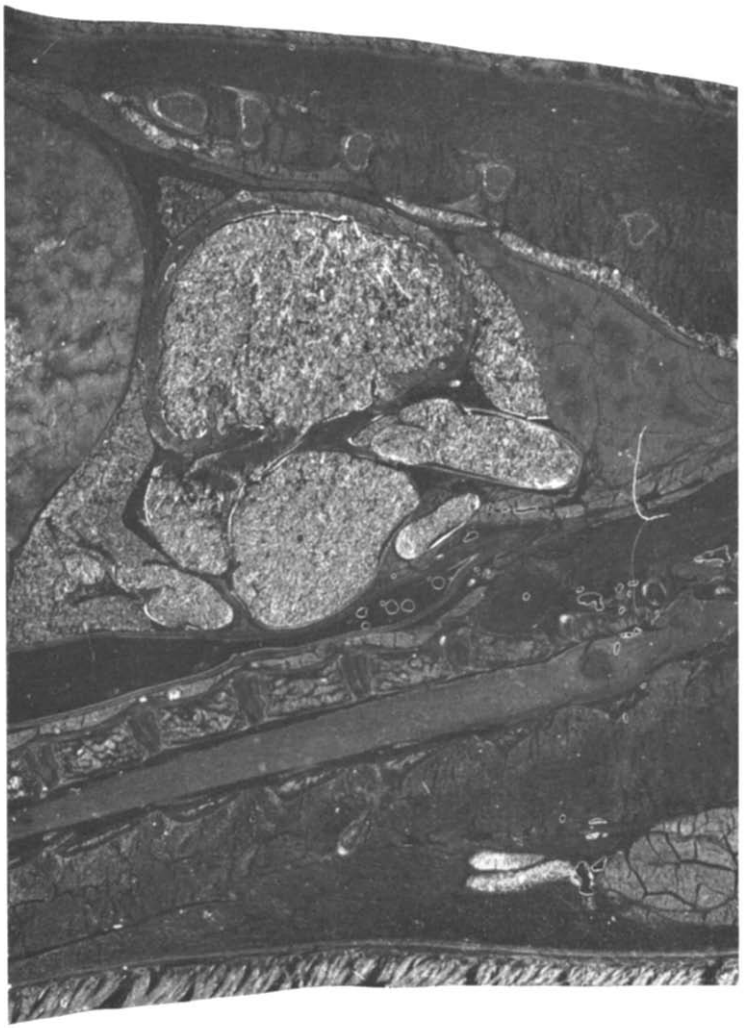

FIG. 8(b)

FIG. 8. Detail of an autoradiogram (A) and the corresponding section (B), obtained from a rat at 15 min after i.v. administration of 10 mg/kg of stercuronium-^{14}C. (See legend to Fig. 5.)

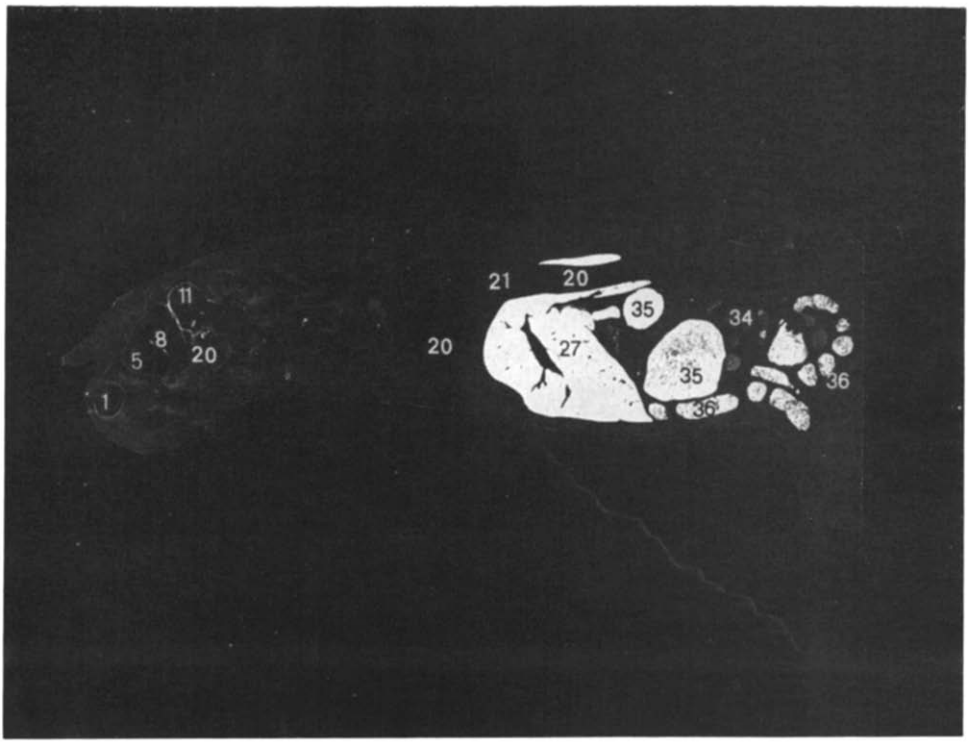

FIG. 12. Distribution of radioactivity in a rat at 24 hr after i.v. administration of 10 mg/kg of stercuronium-^{14}C. (See legend to Fig. 5.)

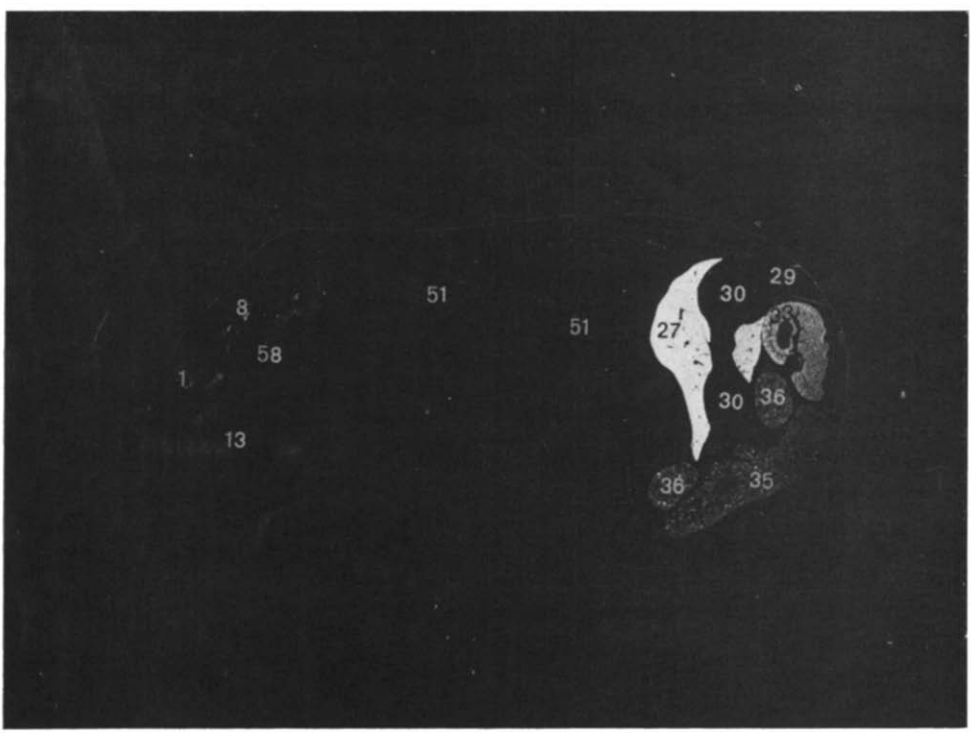

FIG. 13. Distribution of radioactivity in a rat at 120 hr after i.v. administration of 10 mg/kg of stercuronium-^{14}C. (See legend to Fig. 5.)

The kidneys showed an uneven distribution of the radioactivity, the main concentration being observed in the inner cortical zone, composed mainly of the straight portions of the proximal tubules.[6]

At the initial time intervals the lungs showed a level of radioactivity comparable to that in the blood (Figs. 5 and 6). A fairly strong accumulation was observed in connective tissue, notably in muscle fasciae, which stand out as a well-defined pattern in the autoradiograms (Fig. 5).

The connective tissues of skin and hair follicles were radioactive as well. The ribs, in transverse view, showed a ringshaped zone of high radioactivity, not corresponding to the periosteum but to more inner structures (Fig. 8).

In the vertebrae the radioactivity was strongly concentrated in the region of the epiphyseal cartilage, while there was also intravertebral accumulation, probably in the intervertebral disc (Fig. 8).

In the brain no radioactivity was observed with the exception of the choroid plexuses and the meninges.

The eye showed concentration of radioactivity in its membranes, probably the choroid and iris.

Long-term excretion studies

Rats were injected with labelled stercuronium and the excretion of radioactivity by the various routes was monitored as soon as the animals no longer needed artificial respiration. The main route for the elimination of stercuronium radioactivity was found to be the fecal one (Figs. 9 and 10). Ligation of the common bile duct reduced fecal excretion dramatically by $\pm 60\%$ of the injected radioactivity (Fig. 10). This gives an indication of the amount excreted in the feces via the bile.

In the data on the urinary excretion over the first 24-hr period, the loss of radioactivity in urine produced spontaneously during the period of neuromuscular blockade

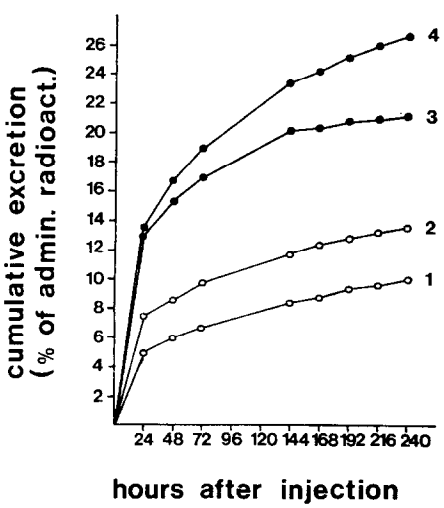

FIG. 9. Cumulative excretion of radioactivity in the urine of normal rats (open circles) and rats with a cholestasis, after i.v. administration of 10 mg/kg of stercuronium-^{14}C.

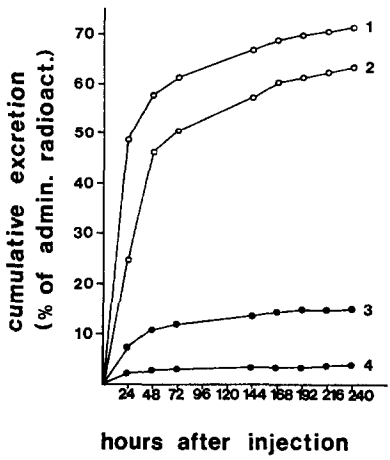

FIG. 10. Cumulative excretion of radioactivity in the feces of normal rats (open circles) and rats with a cholestasis, after i.v. administration of 10 mg/kg of stercuronium-^{14}C.

was ignored (see also the experimental part). Although the information derived from the urinary excretion data of the first 24-hr period thus is not fully reliable, the curves in Figs. 9 and 10 clearly indicate two phases in the excretion course, both in the urine and in the feces. In the period 0–24/48 hr a fairly rapid excretion of the radioactivity occurred; later a much slower and persisting elimination was seen. For some animals semilogarithmic plots of excretion rate against time showed this slow excretion phase to approach first-order kinetics (Fig. 11). A half-life of over 100 hr for the urinary excretion and $\pm$70 hr for the fecal excretion of radioactivity could be derived. The prolonged fecal elimination is obviously related to the prolonged biliary elimination, as can be concluded from Table 2.

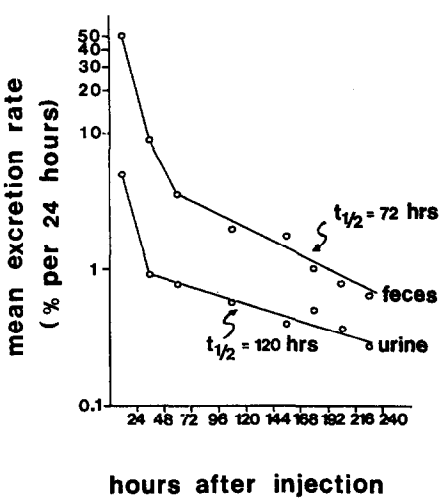

FIG. 11. Semilogarithmic plot of the excretion rate of radioactivity in rat urine and feces, after i.v. administration of 10 mg/kg of stercuronium-^{14}C.

TABLE 2. BILIARY EXCRETION IN BILE FISTULA RATS AT DIFFERENT TIME INTERVALS AFTER INJECTION OF 10 mg/kg OF STERCURONIUM-^{14}C

Rat no.	Interval of bile collection (hr after injection)	Radioactivity recovered (% of radioactivity administered)
1	24–31	1·08
2	48–55	0·44
3	72–79	0·18

In the excretion experiments no radioactivity in the form of $^{14}CO_2$ could be detected; this indicates that the onium group is stable.

Long-term distribution studies

The distribution of the stercuronium radioactivity in rats at 24 and 120 hr after injection is shown in Figs. 12 and 13, respectively. At these later points of time radioactivity was still clearly observable in the liver, intestines, kidneys, choroid plexuses, meninges, and membranes of the eye.

Figure 14 represents a semilogarithmic plot of the time course of the liver concentration, evaluated from the whole-body sections as described in the experimental part. About 24 hr after injection of the labelled stercuronium the concentration of the radioactivity in the liver began to decrease in a process approaching first-order kinetics, with a half-life of ±62 hr.

Biotransformation studies

Figure 15 illustrates the chromatographic analysis of rat urine after injection with stercuronium-^{14}C.

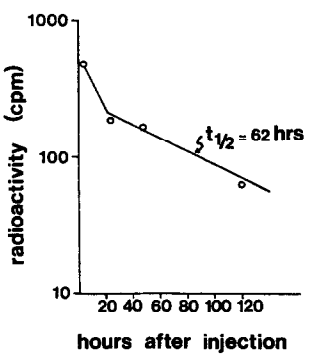

FIG. 14. Semilogarithmic plot of time course of the radioactivity in the rat liver, after i.v. administration of 10 mg/kg of stercuronium-^{14}C. Each point represents the mean of radioactivity determinations in three liver samples.

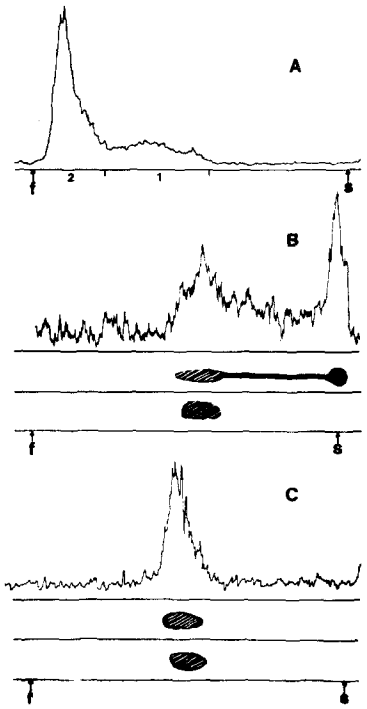

FIG. 15. Chromatographic analysis of the butanol extract of rat urine, collected over a period of 24 hr after i.v. administration of 10 mg/kg of stercuronium-^{14}C. With the butanol 92 per cent of the urinary radioactivity was extracted.

(a) Scan of the radioactivity distribution on the primary chromatogram, obtained with solvent system a.
(b) The upper chromatogram and the scan of its radioactivity distribution refer to the eluate of zone 1 on the primary chromatogram. Unlabelled stercuronium was added as a marker.
The lower chromatogram represents unlabelled stercuronium as a reference substance. Solvent system b.
(c) As (b) but with eluted zone 2 of the primary chromatogram.
The scales of the scans are not directly comparable, since they were taken with different settings of the instrument.

The scan of the primary chromatogram (Fig. 15) showed the principal maximum of the radioactivity at the R_f value of unchanged stercuronium in the solvent system used. However, radioactivity was also present in a rather large area, indicated by 1 in Fig. 15A. Repeated chromatography of an extract of this last region with solvent system b showed part of the radioactivity now concentrated at the R_f value of unchanged stercuronium and corresponding with the coloured spot of the unlabelled stercuronium added as an internal marker. Tailing and retention at the point of application were observed both for the radioactivity and for the internal marker. This suggests the involvement of unaltered stercuronium in these phenomena.

Chromatography of the eluate of area 2 in Fig. 15A with solvent system b again showed only one radioactivity maximum, corresponding with the spot of added, unlabelled stercuronium. In summary, this experiment gives no evidence for the occurrence of stercuronium metabolites. The presence of minor metabolites cannot be

excluded but the main portion of the radioactivity accounted for seems to consist of unchanged stercuronium. This conclusion can be extended to the analytical results obtained with the urine collected at later time intervals and to bile.

Binding to rat serum constituents

In dialysis experiments, performed as described in the experimental part, no significant binding of stercuronium to constituents of rat serum at neutral pH could be demonstrated.

In the same experimental set-up many other drugs of a non-quaternary type showed distinct binding.

DISCUSSION

From the experimental findings the following picture of the fate of stercuronium in the rat emerges. Immediately after injection the compound is distributed throughout the extracellular space and reaches the receptors involved in the neuromuscular blockade. In some of the extracellular compartments, related with connective tissue and structural bone elements, the substance is accumulated compared with the blood concentration, probably because of binding to tissue components like mucopolysaccharides. An uneven distribution in the extracellular space has also been suggested for decamethonium, on the basis of experiments in which it was injected in a [^{14}C] labelled form in the rabbit.[7] The level of stercuronium in the blood and extracellular space rapidly decreases, mainly because of the uptake in the liver, while to a lesser extent the kidneys, too, play a role in the clearance. This rapid clearance from the blood and the extracellular space can be regarded as the factor responsible for the short action of the stercuronium.

The implication of the redistribution is, that nearly all radioactive material has accumulated in the liver and kidneys after the drug has ceased to be active. The mechanism of the uptake in the liver remains unclear. We must assume the radioactivity in the liver to be located intracellularly, because of the strong elimination of the compound in the bile. It is generally accepted, that normally there are no direct connections between bile canniculi and blood vessels. From the literature it is known that with regard to permeability the liver holds a special position among the organs. The membrane of the hepatic parenchymal cells, although of a lipid nature, seems to contain rather large pores which allow to pass a number of lipid insoluble substances, which are withheld by the membranes of the cells of almost any other organ.[8] The autoradiograms show a very pronounced accumulation of radioactivity in the liver. This indicates some mechanism—possibly binding to cell constituents by which the stercuronium molecules are retained.

The differences in half-lives between urinary and fecal excretion of radioactivity observed in the same animal (Fig. 11) suggests that the slow excretion phase is not only governed by a storage pool in the liver, but probably also by a separate storage pool in the kidneys, without any equilibration between the two pools. Moreover, one should not disregard the possibility that both in biliary and urinary excretion of quaternary compounds like stercuronium active transport mechanisms are involved,[9,10] complicating kinetic interpretation of the excretion data.

The main factor involved in the decrease of the storage pool in the liver seems to be biliary excretion, a process which direct experiments showed to be long lasting (Table 2). Distribution studies also confirmed the occurrence of radioactivity in the intestinal tract at even the last time intervals of study (Fig. 13). Another argument is the rather good correspondence between the half-life of the stercuronium radioactivity in the liver ± 62 hr (Fig. 14)—and that of the fecal excretion, which in a separate experiment was found to be of the order of 70 hr (Fig. 11).

There was no evidence for biotransformation of stercuronium in the rat. This is not surprising because quaternary ammonium compounds generally lack the lipophility necessary to penetrate the liver microsomal system, where the major biotransformations occur.

Also with regard to the quaternary curare-like agents *d*-tubocurarine and gallamine triethiodide (Flaxedil®) unchanged excretion in the urine has been reported.[11] The fate of stercuronium in the rat, as it emerges from our data is, however, different from that of these drugs and some other curare-like agents the metabolic fate of which has been described elsewhere. In experiments with tritium-labelled *d*-tubocurarine, gallamine and decamethonium the radioactivity was found to be distributed over a larger volume than with stercuronium, since also the lungs, spleen, and salivary glands showed accumulation of the radioactivity.[12]

The inability of stercuronium to penetrate the brain, except for the choroid plexuses and the meninges (probably the arachnoid) is in agreement with the behaviour of other quaternary ammonium compounds.[13]

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